UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR		ATTORNEY DOCKET NO.
09/593,914	06/14/00	HYLDIG-NIELSEN	J	BP9901US

023544
BOSTON PROBES, INC
75 E WIGGINS AVENUE
BEDFORD MA 01730

HM12/0718

EXAMINER	
MYERS,C	
ART UNIT	**PAPER NUMBER**
1655	9

DATE MAILED: 07/18/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (Rev 11/00)

1- File Copy

Office Action Summary	Application No. 09/593,914	Applicant(s) HYLDIG-NIELSEN ET AL.	
	Examiner Carla Myers	Art Unit 1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 July 2000* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-34,46-49,60-62,72 and 80-85* is/are pending in the application.

4a) Of the above claim(s) *34* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-33,46-49,60-62,72 and 80-85* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15)☒ Notice of References Cited (PTO-892)
16)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17)☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
18)☐ Interview Summary (PTO-413) Paper No(s). _____ .
19)☐ Notice of Informal Patent Application (PTO-152)
20)☐ Other: .

1. ***RESTRICTION***

The following restriction requirement applies to claims 10, 11, 21, 22, 34, 61 and 62. Prior to setting forth the restriction requirement, it is pointed out that Applicants have presented the claims in improper Markush format. See Ex parte Markush, 1925 C.D. 126 and In re Weber, 198 USPQ 334. The claims are improperly joined as the claimed probes are directed to distinct nucleic acid molecules. A reference against one nucleic acid molecule would not be a reference against the other nucleic acid molecule. Therefore, the restriction will be set forth for each of the various groups, irrespective of the improper format of the claims, because the claims do not recite proper species. Upon election, Applicants are required to amend the claims to set forth only the elected inventive groups.

Restriction to one of the following inventions is required under 35 U.S.C. § 121:

I. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 1**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

II. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 2**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

III. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 3**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

IV. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 4**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

V. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 5**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

VI. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 6**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

VII. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 7**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

VIII. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 8**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

IV. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 9**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

X. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 10**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

XI. Claims 10, 11, 21, 22, 34, 61 and 62, drawn to probes and methods of using probes wherein said probes comprise **SEQ ID NO: 11**, classified in Class 435, subclass 6 and Class 536, subclass 24.32.

The inventions are distinct, each from the other because of the following reasons:

Inventions I-XI are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different modes of operation, different functions, or different effects (MPEP § 806.04, MPEP § 808.01). In the instant case the different inventions are drawn to distinct probes each comprising a unique nucleotide sequence and thereby each having unique functional properties. These sequences are thus deemed to constitute independent and distinct inventions within the meaning of 35 U.S.C. 121. Absent evidence to the contrary, each such nucleotide sequence is presumed to represent an independent and distinct invention, subject to a restriction requirement pursuant to 35 U.S.C. 121 and 37 CFR 1.14.

Because these inventions are distinct for the reasons given above and have acquired a different status in the art as demonstrated by their recognized divergent subject matter and

because inventions I-XI require different searches that are not co-extensive, examination of these distinct inventions would pose a serious burden on the examiner and therefore restriction for examination purposes as indicated is proper.

During a telephone conversation with Brian Gildea on April 26, 2001, a provisional election was made with traverse to prosecute the invention of group I, drawn to probes comprising SEQ ID NO: 1. Affirmation of this election must be made by applicant in replying to this Office action. Claim 34 and the subject matter of claims 10, 11, 21, 22, 34, 61 and 62 directed to SEQ ID NO: 2-11 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).

2. The disclosure is objected to because of the following informalities:

In claims 21 and 61, "at least portion" should be amended to read "at least a portion".

The specification is objected to because the assigned SEQ ID NOS have not been used to identify each sequence listed, as required under 37 CFR §1.821(d). See for example, page 29 of the specification.

3. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821-1.825 because the previously submitted Sequence Listing does not include all of the sequences recited in the specification. In particular, the Sequence Listing does not include the sequence set forth on pages 16 and 35. Applicant is required to submit a new CRF and paper copy of the Sequence Listing containing these sequences, in addition to the previously listed sequences, an amendment directing the entry of the Sequence Listing into the specification, an amendment directing the insertion of the SEQ ID NOS into the appropriate pages of the specification and a letter stating that the content of the paper and computer readable copies are the same.

4. Claims 1-33, 46-49, 60-62, 72, 80-85 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-19 are indefinite over the recitation of "suitable for use in an in-situ hybridization assay" because "suitability" is a latent characteristic and the claims do not set forth the criteria by which to determine suitability. That is, it is not clear whether the recited probes have the potential to be used in an in situ hybridization assay only under some unstated conditions or only after the probes have been modified in some unstated manner or if the probes do in fact have the property of being useful as probes in an in situ hybridization assay. This rejection may be overcome by

amendment of the claims to recite, for example, "probe for use in an *in-situ* hybridization assay". Similarly, claims 20-33 are indefinite over the phrase "suitable for detecting".

Claims 1-8 are indefinite over the recitation of "directed to a yeast specific target sequence". The term "directed" has not been clearly defined in the specification and there is no fixed art definition for this term as it applies to a probe. Therefore, it is not clear as to whether the claimed probe hybridizes, specifically or non-specifically, to a yeast specific sequence or whether the probe consists of a yeast specific sequence or whether the probe consists of a sequence which shares some unspecified level of sequence identity with a yeast specific sequence. This rejection may be overcome by amendment of the claims to recite, for example, "probing nucleobase sequence which specifically hybridizes to a yeast specific target sequence".

Claims 5-7 are indefinite over the recitation of "designed to detect" because this phrase does not clearly set forth the structural and/or functional properties of the probe. It is not clear as to whether the probe does in fact detect one or more species of yeast or whether this limitation serves only to define a method of making a probe, but does not define the properties of the resulting probe.

Claims 9-19 are indefinite over the recitation of "and particularly *Dekkera bruxellensis*".The phrase "and particularly" renders the claim indefinite because it is unclear whether the limitations following the phrase are part of the claimed invention. See MPEP § 2173.05(d). That is, it is unclear as to whether the claim is intended to be limited to only probes

which detect *Dekkera bruxellensis* or whether the claims are intended to be limited to probes which detect any *Dekkera/Brettanomyces* yeast.

Claim 11 is indefinite over the phrase "wherein the probing nucleobase sequence is exactly as it appears in the claim". The claim does not specifically set forth a single sequence for the nucleobase, but rather sets forth a sequence consisting of or comprising (a) SEQ ID NO: 1, (b) a sequence fully complementary thereto, (c) a sequence having 90% identity with SEQ ID NO: 1, and (d) a sequence fully complementary to a sequence having 90% identity with SEQ ID NO: 1. Therefore, it is not clear as to what is intended to be encompassed by the "sequence as it appears in the claim". It is also unclear as to what "the claim" refers to. This rejection may be overcome by amendment of the claim to recite, for example, "wherein the probing nucleobase consists of SEQ ID NO: 1 or a sequence fully complementary thereto". Similarly, claims 22 and 62 are indefinite over the recitation of "the probing nucleobases are exactly as represented in the claim".

Claims 23-24 are indefinite over the recitation of "in the same assay" because this phrase lacks proper antecedent basis since the claim does not previously refer to an assay.

Claim 24 is indefinite over the recitation of "the different probes" because this phrase lacks proper antecedent basis since the claim previously refers to a probe set but not to different probes.

Claim 27 is indefinite over the recitation of "probes of the set are labeled" because it is not clear as to whether all of the probes of the set are labeled or whether only some of the probes of the set are labeled.

Claims 46-49, 60-62 and 80-85 are indefinite over the recitation of "suitable *in-situ* hybridization conditions". While the specification discusses a number of different conditions which might constitute suitable conditions and discusses in general the concept of suitable in situ hybridization conditions, the specification does not provide a complete and fixed definition for this phrase. Furthermore, because the claim does not set forth the criteria for determining the suitability of the conditions and therefore it is unclear as to what would constitute suitable conditions. For example, it is unclear as to whether the conditions would allow for any in situ hybridization assay to be performed or if the conditions are specifically designed to allow for the in situ detection of yeast nucleic acids using an enzyme labeled probe.

Claims 72 and 80-82 are indefinite over the recitation of "suitable for performing an assay" because it unclear as to what properties would be required of the kit in order to for it to be considered suitable for performing an assay to detect, identify or enumerate *Dekkera/Brettanomyces* since the claims do not set forth the criteria for determining "suitability". This rejection may be overcome by amendment of the claims to recite "kit for performing an assay...".

Claims 80-82 are indefinite because it is unclear as to whether the recited reagents of a)-h) are considered to be the "reagents or compositions necessary to perform the assay" or whether these reagents are present in addition to the "reagents or compositions necessary to perform the assay". It is also unclear as to whether the soybean labeled probe of e) is present in addition to the "one or more Dekkera/Brettanomyces specific probes" (as recited in a) of claim 80).

Claims 80-82 are indefinite over the recitation of "the enzyme activity of the soy bean peroxidase linked to the peptide nucleic acid probe" because the phrases "the enzyme activity" and "the peptide nucleic acid probe" lack proper antecedent basis.

Claims 83-85 are indefinite over the recitation of "suitable culture media" and "suitable culture conditions" because the claims do not set forth the criteria for determining. That is, it is unclear as to what the culture media and culture conditions are intended to be suitable for.

Claims 83-85 are indefinite over the recitation of "fixing the microcolonies of yeast" because this phrase lacks proper antecedent basis. While the claim previously refers to yeast, the claim does not previously refer to "microcolonies of yeast".

Claims 84 and 85 are indefinite over the recitation of "the kit of claim 83" because claim 83 is directed to a method, rather than a kit.

5. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-7, 9, 12, 14, 15, 20, 23, 24, 26-28, 33, 46-49, 60, 83-85 are rejected under 35 U.S.C. 102(a) as being anticipated by Stender et al (Abstracts of the General Meeting of the American Society for Microbiology, May 30-June 3, 1999, page 516).

It is noted that the authorship of the Stender reference is distinct from the inventorship of the instant application and that this rejection may be overcome by filing a 132 Katz-type Declaration, if appropriate.

Stender discloses *in situ* hybridization methods for the detection and enumeration of *Brettanomyces* species. Stender teaches that the *in situ* hybridization method is performed using peroxidase labeled probes complementary to sequences of the Brettanomyces 26S rRNA region. In the method of Stender, a wine sample is filtered using a membrane to allow for the isolation and separation of microorganisms on the membrane (which membrane thereby is considered to have a pore size which does not allow the *Brettanomyces* yeast to pass through the membrane); the membrane is placed in a culture medium for up to 44 hours; the colonies on the membrane are contacted with a peroxidase labeled PNA probe under conditions suitable for hybridization and fixation; and peroxidase enzyme activity is visualized by detecting a chemiluminescent reaction as indicative of the presence of *Brettanomyces* yeast in the sample. It is stated that each *Brettanomyces* micro-colony is observed as a small dot, which provides a means for simultaneously identifying and enumerating the yeast (i.e., allows for the enumeration of CFU in the sample). Stender further teaches that the probes utilized in the *in situ* hybridization method are specific for particular isolates of *Brettanomyces* as well as for other species of yeast potentially found in wine.

6. Claims 9, 14, 15, 17, 20, 23, 24, 27, 28, 30, 33 and 60 are rejected under 35 U.S.C. 102(b) as being anticipated by Kosse (reference "DF").

Kosse discloses dot blot and *in situ* hybridization methods for the detection and enumeration of *Dekkera bruxellensis*. Kosse teaches that the *in situ* hybridization method is performed using fluorescent labeled probes (e.g., TRITC or FLUOS) and that the dot blot hybridization is performed using digoxigenin labeled probes (page 469). The probes are complementary to sequences of the *Dekkera bruxellensis* 18S rRNA region (page 469; Table 2). The reference teaches that prior to *in situ* hybridization, yeast cell walls must be permeablized and that probes must be selected to yeast 18S rRNA regions which are fully accessible to probes (see page 478). Kosse teaches that *Dekkera bruxellensis* was successfully detected by in situ hybridization using 20% formaldehyde (see Table 2 and page 474). Table 2 lists additional 18S rRNA probes for the detection of other yeasts known to cause spoilage of dairy products (see page 468 and Table 2). Kosse further teaches that it is essential to provide accurate methods for detecting the presence of yeast in dairy products and other foods so as to ensure high quality and safe food products (see page 468).

7. Claims 9-11, 13 are rejected under 35 U.S.C. 102(b) as being anticipated by De Wachter et al (GenBank Accession No. X58052).

De Wachter teaches an isolated nucleic acid consisting of the sequence of the 18S rRNA of *Dekkera/Brettanomyces bruxellensis*. The 18S rRNA of De Wachter comprises the sequence of SEQ ID NO: 1 (see nucleotides 1066-1052 of GenBank Accession No. X58052). The nucleic acid of De Wachter is considered to have the property of being suitable as a probe for the detection, identification or quantitation of *Dekkera/Brettanomyces bruxellensis*.

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Claim 72 is rejected under 35 U.S.C. 103(a) as being unpatentable over Stender et al (1999).

Stender (1999) discloses *in situ* hybridization methods for the detection and enumeration of *Brettanomyces* species. Stender teaches that the *in situ* hybridization method is performed using peroxidase labeled probes complementary to sequences of the Brettanomyces 26S rRNA region. In the method of Stender, a wine sample is filtered using a membrane to allow for the isolation and separation of microorganisms on the membrane (which membrane thereby is considered to have a pore size which does not allow the *Brettanomyces* yeast to pass through the membrane); the membrane is placed in a culture medium for up to 44 hours; the colonies on the membrane are contacted with a peroxidase labeled PNA probe under conditions suitable for hybridization and fixation; and peroxidase enzyme activity is visualized by detecting a

chemiluminescent reaction as indicative of the presence of *Brettanomyces* yeast in the sample. It is stated that each *Brettanomyces* micro-colony is observed as a small dot, which provides a means for simultaneously identifying and enumerating the yeast (i.e., allows for the enumeration of CFU in the sample). Stender further teaches that the probes utilized in the *in situ* hybridization method are specific for particular isolates of *Brettanomyces* as well as for other species of yeast potentially found in wine. Stender does not teach packaging the probes and reagents for in situ hybridization in a kit. However, reagent kits for performing diagnostic methods were conventional in the field of molecular biology at the time the invention was made. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the probes and reagents for performing in situ hybridization in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Brettanomyces*.

9. Claims 8, 13, 16-19, 25, 29-32, and 80-82 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stender (1999) in view of Stender (1998; WO 98/15648; reference BB).

The teachings of Stender are presented above. With respect to claims 1-8, 16 and 29, Kosse teaches dot blot hybridization methods performed using digoxigenin probes and *in situ* hybridization methods performed using fluorescent labeled probes. Kosse does not specifically teach using soy bean peroxidase to label the probes. However, Stender (1998) teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes

(page 20). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of Kosse so as to have specifically labeled the probes with soy bean peroxidase in order to have achieved the benefit of providing an effective means for labeling the probes, thereby facilitating the detection of *Dekkera bruxellensis*.

With respect to claims 18, 19, and 32, Stender (1999) does not teach immobilizing the probes onto a solid support. However, Stender (1998) teaches that hybridization probes may be immobilized onto solid supports and particularly may be in the format of an array (page 31). It is stated that the use of an array provides the advantage of allowing for the simultaneous analysis using 100 or more different probes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have immobilized the probes of Stender (1999) onto a solid support, as taught by Stender (1998), in order to have achieved the benefit of simultaneously assaying for the presence of distinct target sequences complementary to a multitude of different probes.

With respect to claim 25, Stender (1999) does not teach adding blocking probes to the probe sets. However, Stender (1998; pages 25 and 28) teaches adding blocking probes (i.e., random non-selected probes) to hybridization reactions in order to reduce non-specific binding. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of Stender (1999) so as to have included the "blocking probes" disclosed by Stender (1998) in order to have accomplished the objective of reducing non-specific binding of the yeast probes.

With respect to claims 13 and 31, Stender (1999) does not teach probe sets in which all of the probes are unlabeled. However, Stender (1998) teaches that hybridization methods may be performed with unlabeled probes. In such methods, the hybridization complexes between PNA probes and target nucleic acids are detected using antibodies which react specifically with PNA/nucleic acid complexes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of Stender so that all of the probes were unlabeled because the use of unlabeled probes would have allowed for the detection of all probes using antibodies to PNA/target nucleic acid complexes and thereby would have provided a simple and equally effective means for detecting *D. bruxellensis*.

With respect to claims 80-82, Stender does not teach packaging soy bean peroxidase labeled probes, enzyme substrates for peroxidase, wash solutions, fixation solutions, culture solutions, filters and film in a kit. However, the method of Stender (1999) in view of Stender (1998) requires the use of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, culture media, filters and film. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis*.

10. Claim 72 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kosse.

Kosse discloses dot blot and *in situ* hybridization methods for the detection and enumeration of *Dekkera bruxellensis*. Kosse teaches that the *in situ* hybridization method is performed using fluorescent labeled probes (e.g., TRITC or FLUOS) and that the dot blot hybridization is performed using digoxigenin labeled probes (page 469). The probes are complementary to sequences of the *Dekkera bruxellensis* 18S rRNA region (page 469; Table 2). The reference teaches that prior to *in situ* hybridization, yeast cell walls must be permeablized and that probes must be selected to yeast 18S rRNA regions which are fully accessible to probes (see page 478). Kosse teaches that *Dekkera bruxellensis* was successfully detected by in situ hybridization using 20% formaldehyde (see Table 2 and page 474). Table 2 lists additional 18S rRNA probes for the detection of other yeasts known to cause spoilage of dairy products (see page 468 and Table 2). Probes are also disclosed which are specific for all yeasts and for all eukaryotes (Table 2). Kosse further teaches that it is essential to provide accurate methods for detecting the presence of yeast in dairy products and other foods so as to ensure high quality and safe food products (see page 468).

Kosse does not teach packaging the probes and reagents for in situ hybridization in a kit. However, reagent kits for performing diagnostic methods were conventional in the field of molecular biology at the time the invention was made. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the probes of Kosse and reagents for performing dot blot or *in situ* hybridization in a kit for the

expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis*.

11. Claims 1-8, 12, 13, 16, 18, 19, 25, 26, 29, 31, 32, and 46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosse in view of Stender (1998).

The teachings of Kosse are presented above. With respect to claims 8, 16 and 29, Stender (1999) teaches that the *in situ* hybridization method is performed using peroxidase labeled probes complementary to sequences of the Brettanomyces 26S rRNA region. Stender (1999) does not specifically teach using soy bean peroxidase to label the probes. However, Stender (1998) teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of Stender (1999) so as to have specifically labeled the probes with soy bean peroxidase in order to have achieved the benefit of providing an effective means for labeling the probes, thereby facilitating the detection of *Dekkera bruxellensis*.

With respect to claims 17 and 30, Stender (1999) does not teach labeling the probes with a fluorescent moiety. However, Stender (1998) teaches that fluorescent labels and enzyme labels provide equally effective means for labeling probes (page 20). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of Stender (1999) so as to have specifically labeled the probes with fluorescent moieties in

order to have achieved the benefit of providing an equally effective means for labeling the probes, and thereby providing an equally effective means for detecting *Dekkera bruxellensis.*

With respect to claims 18, 19, and 32, Stender (1999) does not teach immobilizing the probes onto a solid support. However, Stender (1998) teaches that hybridization probes may be immobilized onto solid supports and particularly may be in the format of an array (page 31). It is stated that the use of an array provides the advantage of allowing for the simultaneous analysis using 100 or more different probes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have immobilized the probes of Stender (1999) onto a solid support, as taught by Stender (1998), in order to have achieved the benefit of simultaneously assaying for the presence of distinct target sequences complementary to a multitude of different probes.

With respect to claim 25, Stender (1999) does not teach adding blocking probes to the probe sets. However, Stender (1998; pages 25 and 28) teaches adding blocking probes (i.e., random non-selected probes) to hybridization reactions in order to reduce non-specific binding. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of Stender (1999) so as to have included the "blocking probes" disclosed by Stender (1998) in order to have accomplished the objective of reducing non-specific binding of the yeast probes.

With respect to claims 13 and 31, Stender (1999) does not teach probe sets in which all of the probes are unlabeled. However, Stender (1998) teaches that hybridization methods may be

performed with unlabeled probes. In such methods, the hybridization complexes between PNA probes and target nucleic acids are detected using antibodies which react specifically with PNA/nucleic acid complexes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of Stender so that all of the probes were unlabeled because the use of unlabeled probes would have allowed for the detection of all probes using antibodies to PNA/target nucleic acid complexes and thereby would have provided a simple and equally effective means for detecting *D. bruxellensis*.

12. Claims 47, 48 and 80-85 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosse in view of Stender (1998) and further in view of Parton (U.S. Patent No. 5,905,038).

The teachings of Kosse and Stender are presented above. The combined references do no teach filtering the microorganism and culturing the microorganism on a filter prior to performing in situ hybridization.

Parton (col. 1-2) teaches methods for isolating and analyzing samples for the presence of microorganisms wherein the methods comprise filtering a sample containing microorganisms through a membrane filter so as to trap the microorganisms on the filter and then culturing the microorganisms on the filter.

In view of the teachings of Parton, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kosse in view of Stender so as to have isolated the microorganisms by filtration and then cultured the microorganisms on the filter prior to performing in situ hybridization in order to have provided an

effective means for purifying and concentrating the microorganisms and for increasing the number of microorganisms to allow for the formation of colony forming units so as to increase the accuracy and sensitivity of the detection method.

Furthermore, Kosse and Stender do not teach kits comprising filters and culture media. However, the method of Kosse in view of Stender requires the use of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, and film. In view of the teachings of Parton, modification of the method of Kosse and Stender to include a filtration and culturing step would have resulted in a method which further required the use of a filter and culture media. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis*.

13. Claims 14-15, 17, 20-24, 27, 28, 30, 33, 60-62, 72 are rejected under 35 U.S.C. 103(a) as being unpatentable over De Wachter in view of Kosse (reference "DF").

De Wachter teaches an isolated nucleic acid consisting of the sequence of the 18S rRNA of *Dekkera/Brettanomyces bruxellensis*. The 18S rRNA of De Wachter comprises the sequence of SEQ ID NO: 1 (see nucleotides 1066-1052 of GenBank Accession No. X58052). The nucleic acid of De Wachter is considered to have the property of being suitable as a probe for the

detection, identification or quantitation of *Dekkera/Brettanomyces bruxellensis*. De Wachter does not teach labeling the 18S rRNA with a detectable moiety.

Kosse teaches hybridization methods, including dot blot hybridization and in situ hybridization, for the detection of *Dekkera bruxellensis*. Kosse teaches labeling probes with either chemiluminescent labels (e.g., digoxigenin) or fluorescent labels (e.g., TRITC or FLUOS) to facilitate the detection of yeasts and to particularly facilitate the detection of *Dekkera bruxellensis* (see page 469). The reference further exemplifies an 18S rRNA probe specific for *Dekkera bruxellensis* (see Table 2) and probes specific for other yoghurt spoiling yeasts. Kosse teaches that it is essential to provide accurate methods for detecting the presence of yeast in dairy products, such as yoghurt, so as to ensure high quality and safe food products (see page 468).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the oligonucleotide of De Wachter so as to have labeled the oligonucleotide with a chemiluminescent or fluorescent moiety in order to have accomplished the objective of providing labeled probes which could have been used in hybridization assays to detect *Dekkera bruxellensis*. Furthermore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have generated probe sets comprising one or more probes for *Dekkera bruxellensis* or comprising probes for *Dekkera bruxellensis* and probes for other yeast and to have labeled each probe with a different detectable moiety in order to have allowed for the detection and differentiation of multiple types of yeast in dairy products, such as yoghurt.

With respect to claims 60-62, in view of the teachings of Kosse, it would have been further obvious to one of ordinary skill in the art at the time the invention was made to have used the labeled 18S rRNA of De Wachter as a probe under suitable hybridization conditions in order to have facilitated the detection of *Dekkera bruxellensis* in dairy samples.

With respect to claim 72, De Wachter does not teach packaging the 18S rRNA oligonucleotide in a kit. However, as discussed above, Kosse teaches using oligonucleotide probes for the detection of *Dekkera* and teaches the reagents required to perform hybridization assays. Furthermore, reagent kits for performing diagnostic methods were conventional in the field of molecular biology at the time the invention was made. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the *Dekkera bruxellensis* probes and reagents for performing hybridization in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis*.

13. Claims 12, 16, 18, 19, 25, 26, 29, 31 and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over De Wachter in view of Kosse and further in view of Stender et al (1998; reference BB).

The teachings of De Wachter and Kosse are presented above. With respect to claims 12 and 26, the combined references do not teach PNA probes for the detection of *Dekkera bruxellensis*. However, Stender (see, for example, pages 3 and 10-11) teaches PNA probes complementary to rRNA sequences which are useful for the detection of microorganisms.

Stender teaches that PNA probes hybridize to RNA or DNA with a higher affinity and specificity than their nucleic acid counterparts. PNA probes are also more stable due to their resistance to naturally occurring nucleases and proteases. Methods are disclosed for modifying nucleic acid probes so as to incorporate peptide nucleic acid moieties (see, for example, pages 13-14). Stender also teaches that PNA probes can be used in either *in situ* or *in vitro* hybridization methods (page 23). In view of the teachings of Stender, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of De Wachter and Kosse by including peptide nucleic acid moieties in the probes and thereby generating PNA probes, in order to have provided probes with increased affinity and specificity and increased resistance to nucleases and proteases.

With respect to claims 16 and 29, De Wachter and Kosse do not teach labeling the probes with soy bean peroxidase. Stender teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of De Wachter in view of Kosse by using soy bean peroxidase to label the probes in order to have provided an equally effective probe for the detection of *Dekkera bruxellensis.*

With respect to claims 18, 19, and 32, De Wachter and Kosse do not teach immobilizing the *D. bruxellensis* and/or yeast probes onto a solid support. However, Stender teaches that hybridization probes may be immobilized onto solid supports and particularly may be in the format

of an array (page 31). It is stated that the use of an array provides the advantage of allowing for the simultaneous analysis using 100 or more different probes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have immobilized the probes of De Wachter and Kosse onto a solid support, as taught by Stender, in order to have achieved the benefit of simultaneously assaying for the presence of target sequences complementary to a multitude of different probes.

With respect to claim 25, De Wachter and Kosse do not teach adding blocking probes to the probe sets. Stender (page 25, 28) teaches adding blocking probes (i.e., random non-selected probes) to hybridization reactions in order to reduce non-specific binding. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of De Wachter and Kosse so as to have included the "blocking probes" disclosed by Stender in order to have accomplished the objective of reducing non-specific binding of the yeast probes.

With respect to claim 31, De Wachter and Kosse do not teach probe sets in which all of the probes are unlabeled. However, Stender teaches that hybridization methods may be performed with unlabeled probes. In such methods, the hybridization complexes between PNA probes and target nucleic acids are detected using antibodies which react specifically with PNA/nucleic acid complexes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of De Wachter and Kosse so that all of the probes were unlabeled because the use of unlabeled probes would have

allowed for the detection of all probes using antibodies to PNA/target nucleic acid complexes and thereby would have provided a simple and equally effective means for detecting *D. bruxellensis*.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carla Myers whose telephone number is (703) 308-2199. The examiner can normally be reached on Monday-Thursday from 6:30 AM-5:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, W. Gary Jones, can be reached on (703)-308-1152. The fax number for the Technology Center is (703)-305-3014 or (703)-305-4242.

Any inquiry of a general nature or relating to the status of this application should be directed to the receptionist whose telephone number is (703) 308-0196.

Carla Myers

July 11, 2001

Carla Myers

CARLA J. MYERS
PRIMARY EXAMINER